Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY ORIGINAL ARTICLE

# Histamine reduces susceptibility to natural killer cells via down-regulation of NKG2D ligands on human monocytic leukaemia THP-1 cells

Yasuhiro Nagai,[1]* Yukinori Tanaka,[1]* Toshinobu Kuroishi,[1] Ryutaro Sato,[1,2] Yasuo Endo[1] and Shunji Sugawara[1]

[1]*Division of Oral Immunology, Department of Oral Biology, and* [2]*Division of Oral Surgery, Department of Oral Medicine and Surgery, Tohoku University Graduate School of Dentistry, Sendai, Japan*

doi:10.1111/j.1365-2567.2012.03565.x
Received 1 February 2011; revised 16 January 2012; accepted 20 January 2012.
*These authors contributed equally to this work.
Correspondence: Dr S. Sugawara, Division of Oral Immunology, Department of Oral Biology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai, 980-8575, Japan.
Email: s_sugawara@dent.tohoku.ac.jp
Senior author: Shunji Sugawara

## Summary

**Natural killer (NK) group 2D (NKG2D) is a key activating receptor expressed on NK cells, whose interaction with ligands on target cells plays an important role in tumorigenesis. However, the effect of histamine on NKG2D ligands on tumour cells is unclear. Here we showed that human monocytic leukaemia THP-1 cells constitutively express MHC class I-related chain A (MICA) and UL16-binding protein 1 on their surface, and incubation with histamine reduced the expression in a dose-dependent and time-dependent manner as assessed by flow cytometry. Interferon-γ augmented the surface expression of the NKG2D ligands, and this augmentation was significantly attenuated by histamine. The histamine H1 receptor (H1R) agonist 2-pyridylethylamine and H2R agonist dimaprit down-regulated the expression of NKG2D ligands, and activation of H1R and H2R signalling by A23187 and forskolin, respectively, had the same effect, indicating that the histamine-induced down-regulation of NKG2D ligands is mediated by H1R and H2R. Quantitative reverse transcription-PCR showed that mRNA levels of the NKG2D ligands and relevant microRNAs were not significantly changed by histamine. Histamine down-regulated the surface expression of endoplasmic reticulum protein 5, and inhibition of matrix metalloproteinases did not impair this down-regulation, indicating that proteolytic shedding was not involved. Instead, pharmacological inhibition of protein transport and proteasome abrogated it, and histamine enhanced ubiquitination of MICA. Furthermore, histamine treatment significantly reduced susceptibility to NK cell-mediated cytotoxicity. These results suggest that histamine down-regulates NKG2D ligands through the activation of an H1R- and H2R-mediated ubiquitin–proteasome pathway and consequently reduces susceptibility to NK cells.**

**Keywords:** histamine; immune evasion; natural killer cell; NKG2D ligands; ubiquitination

## Introduction

Natural killer (NK) cells are important effectors of the innate immune system, providing early defence against several types of tumours and intracellular microbial infections;[1–3] and are also involved in the adaptive immune system.[4] Natural killer cell-mediated cytotoxicity is controlled by activating and inhibitory receptors and NK group 2D (NKG2D) is a key activating receptor expressed on NK, activated $CD8^+$ and $\gamma\delta$ T cells.[1–3]

Abbreviations: ERp5, endoplasmic reticulum protein 5; FU, fluorescence units; H1R, histamine H1 receptor; HDC, histidine decarboxylase; IFN, interferon; mAb, monoclonal antibody; MFI, mean fluorescence intensity; MIC, MHC class I-related chain; MMP, matrix metalloproteinase; NK, natural killer; NKG2D, NK group 2D; PBMC, peripheral blood mononuclear cells; RT, reverse transcription; SD, standard deviation; TNF, tumour necrosis factor; ULBP, UL16-binding protein.

Human NKG2D associates with the adaptor protein, DNAX-activating protein of molecular weight 10 000, its engagement ultimately leading to calcium influx and cytotoxicity.[3]

There are multiple ligands for NKG2D, all of which are homologues of MHC class I proteins. The first ligands identified were MHC class I-related chain (MIC) A and MICB, which are expressed in normal intestinal epithelium and in carcinomas of many tissues.[5] The expression of MICA/B can be increased by culture in interferon-α (IFN-α) and interleukin-15 from dendritic cells.[6] The second family of human NKG2D ligands is the UL16-binding protein (ULBP) family, ULBP1–4, which were first identified as ligands for the human cytomegalovirus protein UL16 with homology to mouse NKG2D ligands, the retinoic acid early transcript 1 family.[7]

Histamine is a bioamine with multiple physiological activities.[8] Histamine is released from stimulated mast cells or basophils. In addition, it is synthesized by histidine decarboxylase (HDC) in non-mast cells and released without being stored.[9–11] HDC is expressed in various organs and tissues in response to a variety of stimuli, including bacterial products, such as lipopolysaccharide and pro-inflammatory cytokines.[10,12,13] Histamine receptors (H1R, H2R, H3R and H4R) belong to the G-protein-coupled receptor superfamily.[8] H1R and H2R are expressed in various cell types, whereas the expression of H3R and H4R is restricted to the brain and haematopoietic cells, respectively. H1R is linked to the activation of phospholipase C through $G\alpha_{q/11}$ protein, which causes the mobilization of intracellular calcium, and H2R is linked to adenylate cyclase and phosphoinositide second messenger systems.[8] H2R-dependent effects of histamine are predominantly mediated by cAMP.

Histamine is an important mediator not only in allergic reactions but also in a variety of immune responses, including the production of pro-inflammatory cytokines and the modulation of the helper T-cell balance.[8] Histamine can synergistically augment inflammatory stimuli such as tumour necrosis factor-α (TNF-α), interleukin-1 and lipopolysaccharide.[14] Histamine does not directly affect the cytotoxicity of NK cells but protects NK cells from inhibition of monocyte-derived reactive oxygen species.[15] The mechanism of this protection is the ability of histamine to maintain the expression of NKG2D and NKp46, a member of the family of natural cytotoxicity receptors.[16]

Protein expression and activity of HDC are increased in experimental and human tumours, such as melanoma,[17,18] breast cancers[19,20] and small cell lung carcinoma.[21] Tumour cells express H1R, H2R and H4R,[22] and histamine is implicated as an autocrine growth factor.[18,23] However, the effect of histamine on NKG2D ligands expressed on tumour cells is unclear. In this study, we investigated whether histamine modulates human NKG2D ligands, MICA, MICB and ULBP1, expressed by human monocytic leukaemia THP-1 cells and NK cell-mediated cytotoxicity.

## Materials and methods

### *Reagents*

Human IFN-γ was obtained from BioLegend (San Diego, CA). Dimaprit was obtained from Wako Pure Chemical Industries (Osaka, Japan). The calcium ionophore A23187, GM6001 and cytochalasin B were obtained from Calbiochem (San Diego, CA,). Golgiplug (containing 10% Brefeldin A) was obtained from BD (Franklin Lakes, NJ). Bortezomib was obtained from Nacalai (Kyoto, Japan). All other reagents were obtained from Sigma-Aldrich (St Louis, MO), unless indicated otherwise.

### *Cells and cell culture*

The human monocytic leukaemia cell line THP-1[24] was obtained from the Cell Resource Centre for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan) and grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal calf serum (Tissue Culture Biologicals, Tulare, CA). THP-1 cells ($2 \times 10^5$ cells/ml) were cultured in 24-well plates in 1 ml of the medium with histamine and other reagents.

### *Flow cytometry*

Cells were incubated with Alexa 488-conjugated anti-human MICA/B monoclonal antibody (mAb) 6D4 (mouse IgG1) (BioLegend), anti-human MICB mAb 236511 (mouse IgG2b), anti-human ULBP1 mAb 170818 (mouse IgG2a) (R&D Systems, Minneapolis, MN) or control IgG on ice for 30 min. Cells stained with anti-MICB, anti-ULBP1 or control IgG were further incubated with FITC-conjugated affinity purified goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) on ice for 30 min. Cells were also stained with rabbit anti-endoplasmic reticulum protein 5 (ERp5) polyclonal antibody (Affinity BioReagents, Golden, CO) or control IgG and further incubated with FITC-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories) on ice for 30 min. Flow cytometric analyses were performed with FACSCalibur and CELLQUEST (BD), and mean fluorescence intensity (MFI) was evaluated. The % reduction of MFI by histamine was calculated as (MFI in the presence of histamine − control MFI)/(MFI in the absence of histamine − control MFI) × 100 (%).

For intracellular staining, cells were treated with Cytofix/Cytoperm (BD) for 20 min and 0·05% Tween-20 for 10 min on ice, and then stained with antibodies.

*Quantitative reverse transcription-PCR*

Quantitative reverse transcription (RT-) PCR was performed as described previously.[25] The primers used for PCR were as follows: MICA, forward 5′-GGA TGG ATC TGT GCA GTC-3′ and reverse 5′-CGT TCC CTG TCA AGT CTC T-3′; MICB, forward 5′-TGT TTC TGC TGC TAT GCC AT-3′ and reverse 5′-CTG ACA TCA GAG GCT GAA ATC-3′; ULBP1, forward 5′-GCA GAG GAT CTT GGC AGT TC-3′ and reverse 5′-CCC CAG TGA AAT CTT CTG GA-3′; and $\beta$-actin, forward 5′-GCA AAG ACC TGT ACG CCA AC-3′ and reverse 5′-CTA GAA GCA TTT GCG GTG GA-3′ (Nihon Gene Research Labs, Sendai, Japan). The PCR conditions were 40 cycles of 95° for 10 s, 68° for 10 s and 72° for 10 s for MICA, 35 cycles of 95° for 10 s, 68° for 10 s and 72° for 10 s for MICB, 35 cycles of 95° for 10 s, 64° for 10 s and 72° for 10 s for ULBP1, and 35 cycles of 95° for 10 s, 60° for 10 s and 72° for 10 s for $\beta$-actin. The product sizes for MICA, MICB, ULBP1 and $\beta$-actin were 167, 182, 161 and 260 bp, respectively. Quantitative RT-PCR for microRNAs was performed as described elsewhere.[26] Total RNA was polyadenylated with poly(A) polymerase and then reverse-transcribed with Moloney murine leukaemia virus reverse transcriptase and a unique Oligo-dT Adaptor primer from the All-in One™ microRNA qRT-PCR Detection kit according to the maunufacturer's instructions (GeneCopoeia, Rockville, MD). The reaction primers were a 3′ adaptor primer and primers based on the microRNA sequences as described.[26] The mRNA levels of the relevant miR-20a and miR-93 and irrelevant miR-103 were normalized to the control miR-16 level and expressed as relative units versus medium alone. The specificity of the PCR was confirmed by the molecular weight of the products and a melting curve analysis for each data point.

*TNF-α release assay*

THP-1 cells were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) in the presence (10 or 50 μM) or absence of GM6001 for 24 hr. The supernatant was then collected, and the amount of TNF-α that it contained was measured using a human TNF-α ELISA kit (BioLegend).

*Phagocytosis assay*

Phagocytosis was assessed through the uptake of pHrodo *Escherichia coli* BioParticles (Invitrogen). To elicit the response, the particles were first opsonized with 25% normal human serum for 30 min at 37°. THP-1 cells were pre-incubated with (1 μg/ml) or without cytochalasin B for 1 hr at 37°. Then, the opsonized particles were added to the culture medium at a final concentration of 20 μg/ml. After 8-hr incubation, the fluorescence from ingested particles was analysed with LSRFORTESSA and DIVA software (BD), and the percentage of pHrodo-positive cells was evaluated.

*Western blotting and immunoprecipitation*

Cells ($10^6$ cells) were lysed with a buffer (100 μl) containing 1% Triton X-100, 50 mM Tris–HCl (pH 7·4), 25 mM *N*-ethylmaleimide, 1 mM PMSF and 10 μg/ml soybean trypsin inhibitor for 30 min at 4°. After SDS–PAGE, gel proteins were electrophoretically transferred to a PVDF membrane (Bio-Rad Laboratories, Hercules, CA). The blot was blocked for 1 hr with 3% (weight/volume) skim milk and 0·05% Tween-20 in PBS and incubated with mouse anti-ubiquitin mAb P4D1 (Santa Cruz Biotechnology, Santa Cruz, CA) at 0·5 μg/ml or rabbit anti-$\beta$-actin polyclonal antibody (BioLegend) at 1 : 2000 in 0·05% Tween-20 in PBS overnight at 4°. Horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG (Pierce Biotechnology, Rockford, IL) were used, respectively, as the second antibodies. After being washed, the blot was visualized with SuperSignal West Femto Maximum Sensitivity substrate (Pierce Biotechnology) in a Chemi Imager (Alpha Innotech, San Leadron, CA). The relative molecular mass of the proteins was estimated by comparison with the position of protein standards (Bio-Rad Laboratories). Band intensities were quantified using IMAGE J software (National Institutes of Health, Bethesda, MD).

For immunoprecipitation, lysates were incubated with protein G-agarose and goat anti-MICA/B polyclonal antibody (Santa Cruz Biotechnology). The samples were washed five times with lysis buffer and subjected to Western blotting with mouse anti-ubiquitin mAb P4D1 or rabbit anti-MICA polyclonal antibody (Abcam, Cambridge, MA).

*Cytotoxicity assay*

Human peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood of healthy volunteers by Lympholyte-H (Cedarlane Laboratories, Hornby, ON, Canada) gradient centrifugation at 800 ***g*** for 20 min at room temperature. The isolated PBMC were washed three times with PBS. NK cells were magnetically purified from the PBMC using human NK cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The PBMC and purified NK cells were used as effector cells.

The cytotoxicity assay was performed using a fluorescent probe as described previously[27] with slight modifications. Briefly, target cells were labelled with 2 μg/ml calcein AM (Molecular Probes, Eugene, OR) for 15 min at 37° in PBS and washed three times with RPMI-1640 medium with 10% fetal calf serum. Labelled target cells ($5 \times 10^4$ cells/well) were incubated in a total volume of 200 μl with effector cells in 96-well round-bottomed

plates. In some experiments, calcein AM-labelled THP-1 cells were pre-incubated with anti-MICA/B mAb 6D4 (mouse IgG2a) (BioLegend) and/or anti-ULBP1 mAb MM0592-10K10 (mouse IgG2) (Abcam), or control mouse IgG, 5 μg/ml each, for 30 min at 37° and used as targets. To achieve total lysis, cells were incubated with 0·1% Triton X-100. After a 4-hr incubation period, plates were centrifuged, and the supernatants were removed and replaced with PBS. Fluorescence units (FU) were measured in a Microplate Reader (Corona Electric, Hitachinaka, Japan). The excitation and emission wavelengths were 490 and 515 nm, respectively. The % viability was calculated as (FU of targets incubated with effector cells − FU of targets incubated with Triton X)/(FU of targets incubated with medium alone − FU of targets incubated with Triton X) × 100 (%), and then the % cytotoxicity was calculated as 100 – % viability. The Ethical Review Board of Tohoku University Graduate School of Dentistry approved the experimental procedures.

### *Statistical analysis*

Experimental values were expressed as means ± standard deviation (SD). The statistical significance of differences was evaluated using an unpaired *t*-test with Welch's correction when comparing two groups, and a one-way analysis of variance using the Bonferroni or Dunnett method when comparing three or more groups. Values of $P < 0{\cdot}05$ were considered to be statistically significant.

## Results

### Down-regulation of surface expression of NKG2D ligands on THP-1 cells by histamine

We first examined the effect of histamine on the expression of NKG2D ligands on human monocytic leukaemia THP-1 cells. THP-1 cells constitutively expressed MICA/B and ULBP1 on the cell surface as assessed by flow cytometry (Fig. 1a). Incubation with histamine at 1 μM for 24 hr resulted in a decrease in the expression of these ligands, and further reductions at 10 and 100 μM of histamine. The expression of MICA/B was significantly decreased at 8 hr and further decreased at 24 and 48 hr (Fig. 1b). The expression of ULBP1 was significantly decreased at 24 and 48 hr. Flow cytometric analysis with MICB-specific mAb showed the expression of MICB to be marginal and not significantly decreased by histamine treatment (Fig. 1c), indicating that THP-1 cells express MICA, but not MICB, on their surface and that MICA expression was down-regulated by histamine.

As NK cells are a major source of IFN-γ,[1] we next examined the effect of IFN-γ on the expression of NKG2D ligands and its histamine-induced down-regulation. Treatment of THP-1 cells with IFN-γ markedly

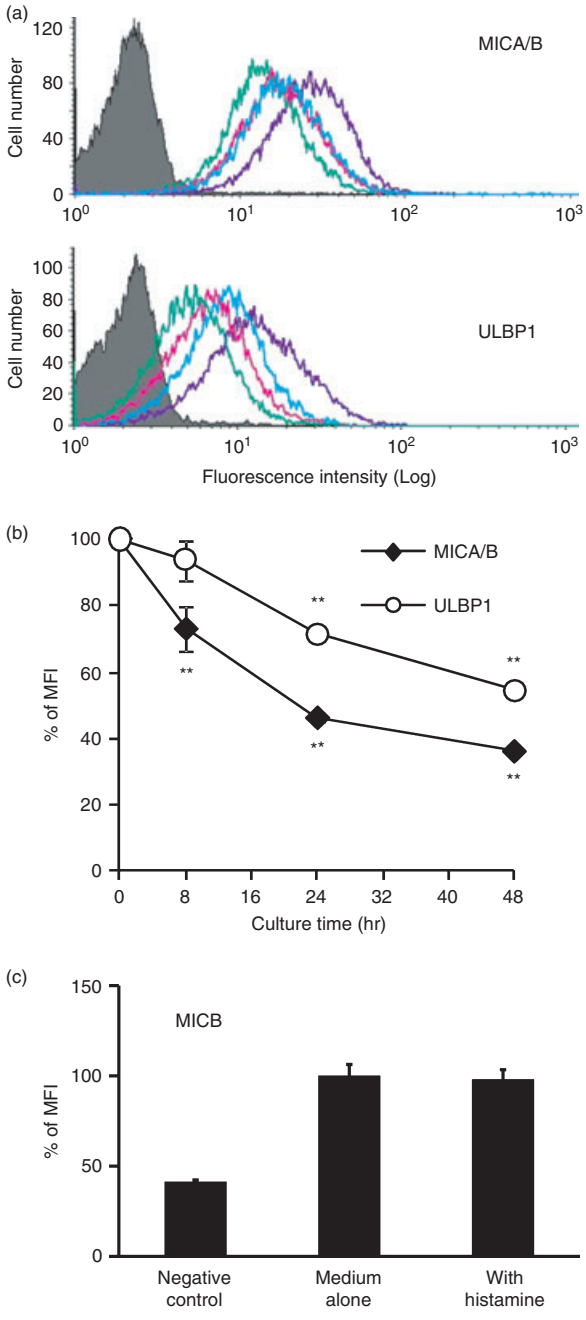



**Figure 1.** Down-regulation of natural killer group 2D (NKG2D) ligand expression by histamine. (a) THP-1 cells were incubated with medium alone (purple) or histamine (1, blue; 10, pink; and 100 μM, green) for 24 hr at 37°. Cells were then stained with anti-MHC class I related chain A/B (MICA/B) or anti-UL16-binding protein 1 (ULBP1) monoclonal antibody (mAb) and analysed by flow cytometry. Grey shows the negative control. (b) THP-1 cells were incubated with 100 μM histamine for the time indicated. The % mean fluorescence intensity (MFI) for triplicate cultures was evaluated on the basis of the value obtained with untreated cells. $^{**}P < 0{\cdot}01$ compared with 0 hr. (c) THP-1 cells were incubated with medium alone or 100 μM histamine for 24 hr at 37°. Cells were then stained with anti-MICB mAb and analysed by flow cytometry. The MFI for triplicate cultures was based on the value obtained with untreated cells.

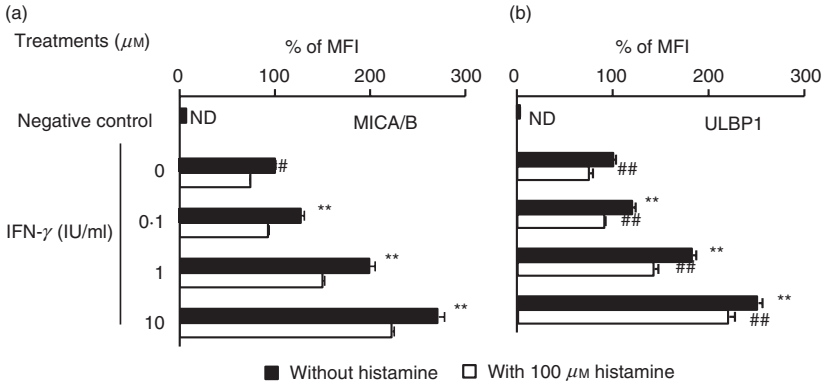



**Figure 2.** Effect of histamine on interferon-$\gamma$ (IFN-$\gamma$)-induced expression of natural killer group 2D (NKG2D) ligands. THP-1 cells were incubated with the indicated concentrations of IFN-$\gamma$ in the presence or absence of histamine (100 μM) for 24 hr. Cells were then stained with anti-MHC class I related chain A/B (MICA/B) (a) or anti-UL16-binding protein 1 (ULBP1) monoclonal antibody (mAb) (b) and analysed by flow cytometry. The % mean fluorescence intensity (MFI) for triplicate cultures was evaluated on the basis of the value obtained with untreated cells. $^{**}P < 0{\cdot}01$ compared with untreated THP-1 cells (medium alone). $^{\#\#}P < 0{\cdot}01$ compared with medium or IFN-$\gamma$ alone. ND, not done.

augmented the expression of MICA/B and ULBP1 in a dose-dependent manner, and the effect of IFN-$\gamma$ at doses examined (0·1–10 IU/ml) was significantly reduced by histamine (Fig. 2).

## Down-regulation of surface expression of NKG2D ligands through H1R and H2R

We previously showed that THP-1 cells constitutively express H1R and H2R at the mRNA and protein levels.[14] Therefore, we next examined whether H1R or H2R was involved in the histamine-induced down-regulation of NKG2D ligand expression. To this end, the H1R agonist 2-pyridylethylamine and the H2R agonist dimaprit were used instead of histamine. Histamine at 100 μM was used as a control. The results showed that levels of MICA/B and ULBP1 were significantly reduced by the H1R or H2R agonist in a dose-dependent manner (Fig. 3a).

H1R is coupled mainly to the $G\alpha_{q/11}$ family of G-proteins that cause the mobilization of intracellular calcium, and H2R is coupled to the adenylate cyclase second messenger system. Therefore, we next examined the effect of a calcium ionophore, A23187, and an adenylate cyclase activator, forskolin, on the expression of the NKG2D ligands. A23187 and forskolin significantly reduced the expression of MICA/B and ULBP1 (Fig. 3b). These results indicate that histamine down-regulates the expression of NKG2D ligands through H1R and H2R on THP-1 cells.

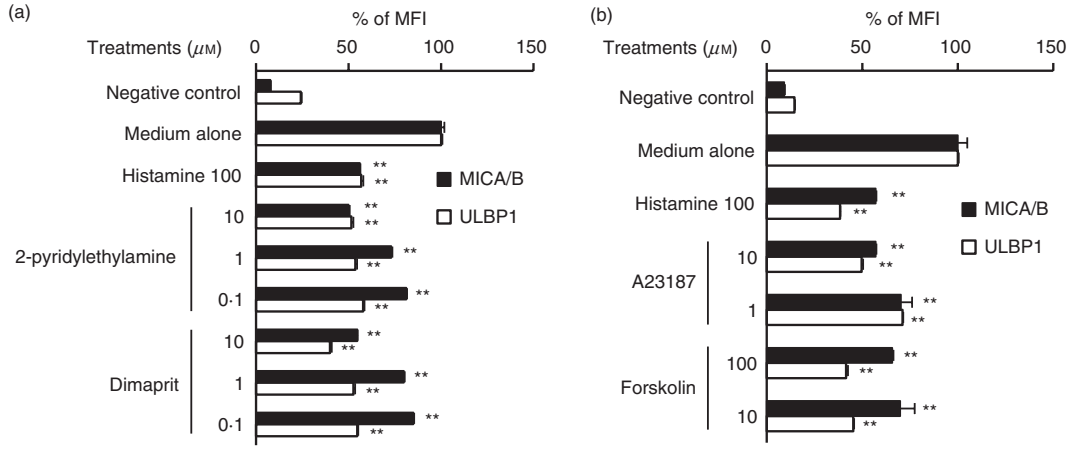



**Figure 3.** Down-regulation of surface expression of natural killer group 2D (NKG2D) ligands through histamine 1 receptor (H1R) and H2R. THP-1 cells were incubated with the indicated concentrations of histamine receptor agonists for 24 hr (a) or with the indicated concentrations of A23187 or forskolin for 24 hr (b). Histamine at 100 μM was used as a control. Cells were then stained with anti-MHC class I related chain A/B (MICA/B) or anti-UL16-binding protein 1 (ULBP1) monoclonal antibody (mAb) and analysed by flow cytometry. The % mean fluorescence intensity (MFI) for triplicate cultures was evaluated on the basis of the value obtained with untreated cells. $^{**}P < 0{\cdot}01$ compared with untreated THP-1 cells (medium alone).

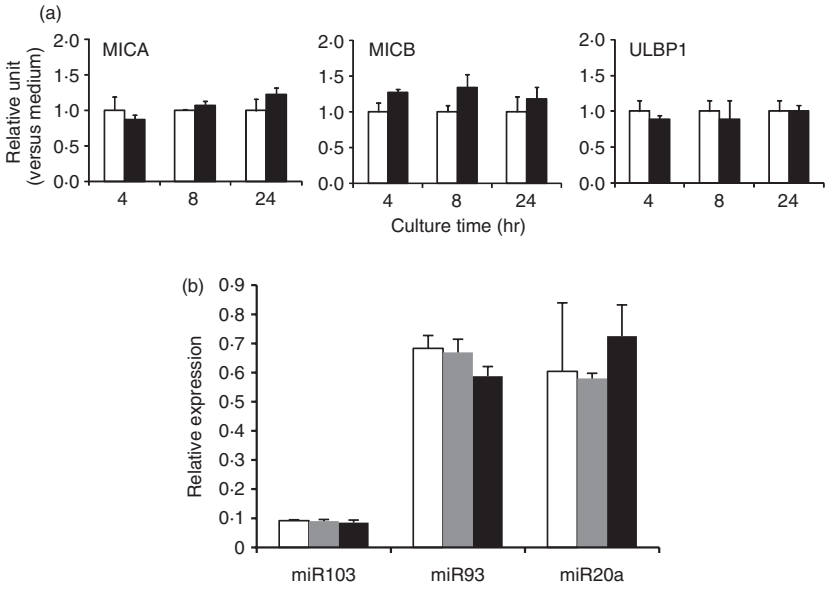



**Figure 4.** Transcriptional regulation or microRNA is not involved in the histamine-induced down-regulation of natural killer group 2D (NKG2D) ligands. (a) THP-1 cells were incubated with medium alone (□) or 100 μM histamine (■) for the time indicated at 37°. The mRNA levels of MHC class I related chain A (MICA), MICB and UL16-binding protein 1 (ULBP1) were determined by quantitative reverse transcription-PCR. The results were normalized by the $\beta$-actin level and expressed as relative units versus medium alone. The results are expressed as the mean ± SD for triplicate assays. (b) THP-1 cells were incubated with medium alone (□) or 100 μM histamine for 8 hr (▩), for 24 hr (■) at 37°. The mRNA levels of relevant miR-93 and miR-20a as well as irrelevant miR-103 were determined by quantitative reverse transcriptase-PCR. The results were normalized by control miR16 level and expressed as relative units versus medium alone. The results are expressed as the mean ± SD for triplicate assays.

### Transcriptional regulation, microRNAs, proteolytic shedding, or internalization is uninvolved in the histamine-induced down-regulation of NKG2D ligands

To explore the mechanism of down-regulation, we examined the mRNA levels of NKG2D ligands in THP-1 cells after histamine treatment. The results of quantitative RT-PCR showed that mRNA levels of MICA, MICB and ULBP1 were not significantly changed by histamine treatment for 4, 8 and 24 hr compared with untreated cells (Fig. 4a), indicating that the histamine-induced down-regulation is post-transcriptional. Human microRNAs can down-regulate MICA and MICB protein expression through inhibition of translation.[26] However, expression of relevant microRNAs (miR-93 and miR-20a) as well as the irrelevant microRNA miR-103 was not significantly changed by histamine treatment for 8 and 24 hr compared with untreated cells (Fig. 4b), suggesting that these microRNAs were not involved in the down-regulation.

The NKG2D ligands were proteolytically shed from tumour cells by matrix metalloproteinases (MMPs), such as TNF-α-converting enzyme,[28–30] and surface ERp5, a protein disulphide isomerase, enabled the shedding of NKG2D ligands.[31] Therefore, we next examined this possibility. However, histamine treatment also down-regulated surface expression of ERp5 (Fig. 5a), and GM 6001, a potent broad-spectrum inhibitor of MMPs, did not impair the histamine-induced down-regulation of MICA/B (Fig. 5b), whereas the release of TNF-α from THP-1 cells induced by PMA and ionomycin was clearly inhibited by GM6001 at the same concentrations (Fig. 5c). Furthermore, soluble forms of MICA and MICB were not detectable in THP-1 cell culture supernatants even after histamine treatment as assessed by ELISA (data not shown). These results indicate that proteolytic shedding of NKG2D ligands is not involved in the down-regulation. Furthermore, inhibition of actin polymerization by cytochalasin B[32] did not impair the down-regulation of MICA/B on THP-1 cells induced by 100 μM of histamine (Fig. 5d), whereas the phagocytosis of *E. coli* BioParticles was clearly inhibited by cytochalasin B at the same concentration (Fig. 5e). These results indicate that internalization of NKG2D ligands is also not involved in the down-regulation.

### Involvement of protein trafficking and ubiquitin-proteasome system in the histamine-induced down-regulation of NKG2D ligands

We next compared total levels (i.e. cell surface and cytoplasm) of MICA/B with cell surface MICA/B by flow cytometry. THP-1 cells were permeabilized before staining with anti-MICA/B to detect total MICA/B. MICA/B expression of untreated (surface) and permeabilized THP-1 cells (total) was comparable, and histamine induced a

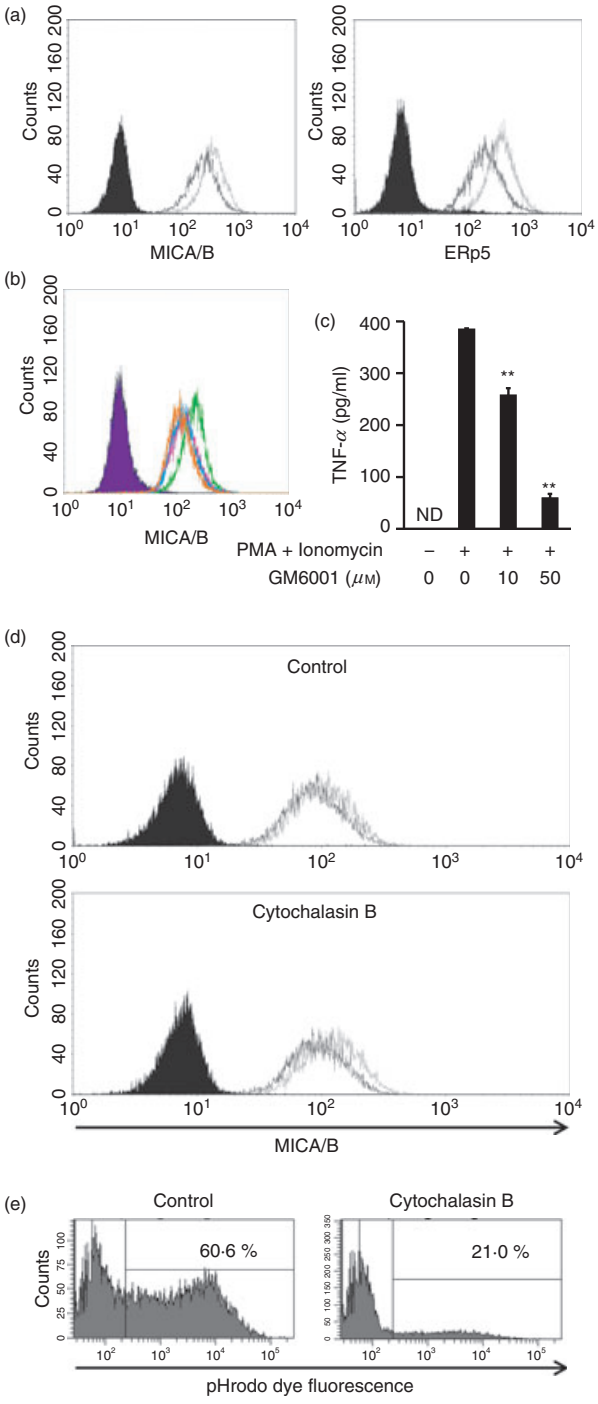


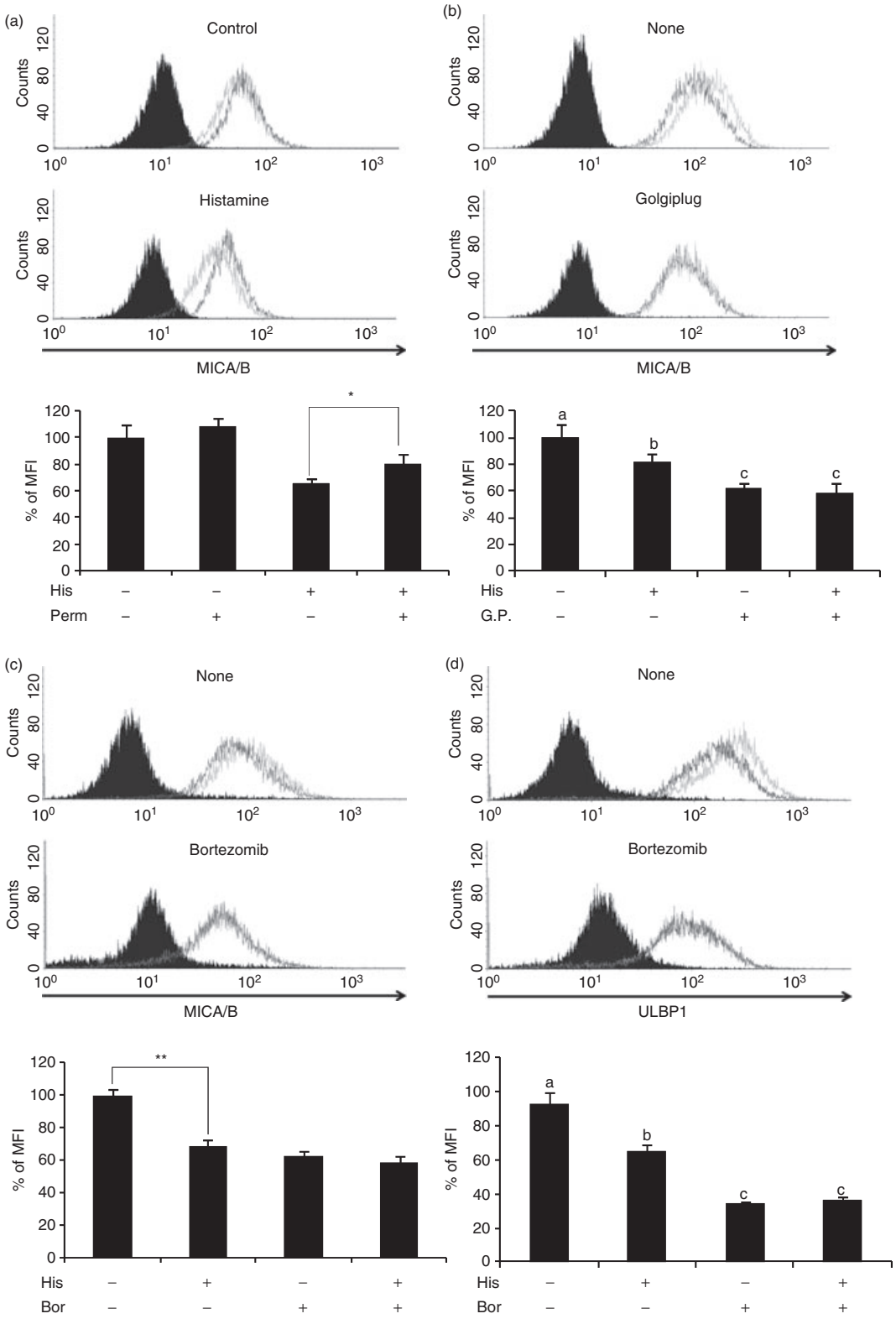

**Figure 5.** Proteolytic shedding or internalization is not involved in the histamine-induced down-regulation of natural killer group 2D (NKG2D) ligands. (a) THP-1 cells were incubated with medium alone (thin line) or 100 μM histamine (thick line) for 24 hr at 37°. Cells were then stained with anti-MHC class I related chain A/B (MICA/B) or anti-endoplasmic reticulum protein 5 (ERp5) monoclonal antibody (mAb) and analysed by flow cytometry. The negative control is represented as black. (b) THP-1 cells were incubated with medium alone (green), 100 μM histamine (pink), or 100 μM histamine in the presence of GM6001 at 10 (blue) or 50 μM (orange) for 24 hr. Cells were then stained with anti-MICA/B and analysed by flow cytometry. Purple shows the negative control. (c) THP-1 cells were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) in the presence (10 or 50 μM) or absence of GM6001 for 24 hr. Tumour necrosis factor-α (TNF-α) levels in the supernatants were measured by ELISA. $^{**}P < 0{\cdot}01$ compared with PMA and ionomycin alone. ND, not detected. (d) THP-1 cells were left untreated (thin line) or incubated with 100 μM histamine (thick line) in the absence (upper panel) or presence of 1 μg/ml of cytochalasin B (lower panel) for 8 hr. Cells were then stained with anti-MICA/B and analysed by flow cytometry. Black represents the negative control. (e) THP-1 cells were pre-incubated with (1 μg/ml) or without cytochalasin B for 1 hr. Then, opsonized pHrodo *Escherichia coli* BioParticles were added to the culture medium at 20 μg/ml. After an 8-hr incubation, the phagocytosis of the particles was analysed by flow cytometry. One representative experiment out of three is shown.

decrease in the total level in permeabilized THP-1 cells as well as the surface expression (Fig. 6a). However, the cell surface MICA/B was significantly lower than total MICA/B in the permeabilized cells. Treatment of THP-1 cells with brefeldin A, a protein transport inhibitor in the endoplasmic reticulum,[33] abrogated the histamine-induced down-regulation of MICA/B expression on THP-1 cells (Fig. 6b). These results suggest that histamine induces MICA/B degradation in cytoplasm and attenuates the surface expression via the endoplasmic reticulum.

The ubiquitin–proteasome pathway plays an essential role in regulating intracellular protein levels,[34,35] and NKG2D ligands can be ubiquitinated.[36,37] Therefore, we next examined this possibility. In the presence of bortezomib, a selective and potent inhibitor of the proteasome,[34,35] histamine did not down-regulate MICA/B (Fig. 6c) and ULBP1 expression (Fig. 6d) as compared with bortezomib alone, although the expression of these ligands was reduced, perhaps as the result of the cytotoxic effect of bortezomib itself.[34,35] In the presence of bortezomib, ubiquitination with high molecular weight was augmented by histamine at 8 and 12 hr (Fig. 7a), indicating that poly-ubiquitination of cellular protein was enhanced by histamine. Furthermore, in the presence of bortezomib, MICA was ubiquitinated and the ubiquitination was enhanced by histamine (Fig. 7b). As MICA is a glycoprotein with molecular weight 65 000–75 000,[28] proteins with lolecular weights > 65 000 were quantified. These results suggest that histamine signalling activated the ubiquitin–proteasome system in THP-1 cells and degraded NKG2D ligands in the cells and consequently reduced the expression on the cell surface.

### Histamine treatment reduces susceptibility to NK cells

We finally examined whether the histamine-induced down-regulation of NKG2D ligands on THP-1 cells correlates with susceptibility to NK cell-mediated cytotoxicity. Freshly isolated PBMC and purified NK cells were




tested for killing of THP-1 cells. Untreated THP-1 cells showed substantial susceptibility to PBMC and NK cells (Fig. 8). Histamine led to a significant decrease in susceptibility of THP-1 cells to PBMC for 48-hr treatment at effector to target (E : T) ratios of 20 : 1 and 10 : 1 (Fig. 8a) and a marked decrease in this susceptibility for 72-hr treatment at E : T ratios tested except for 1·25 : 1 (Fig. 8b). Histamine also induced a significant decrease in

**Figure 6.** Involvement of protein trafficking and proteasome system in the histamine-induced down-regulation of natural killer group 2D (NKG2D) ligands. (a) THP-1 cells were incubated with medium alone (upper panel) or 100 μM of histamine (middle panel) for 24 hr at 37°. Cells were left untreated (thin line) or permeabilized (thick line) and stained with anti-MHC class I-related chain A/B (MICA/B) and analysed by flow cytometry. Black represents the negative control. The % mean fluorescent intensity (MFI) for triplicate cultures was evaluated on the basis of the value obtained with untreated cells (lower panel). His and Perm represent histamine-treated and permeabilized, respectively. $^{*}P < 0{\cdot}05$ between two values. (b–d) THP-1 cells were incubated with medium alone (thin line) or 100 μM histamine (thick line) in the absence (upper panel) or presence of Golgiplug (containing 10% Brefeldin A) at 1 : 1000 (middle panel) for 8 hr (b), or in the absence (upper panel) or presence of 500 nM of bortezomib (middle panel) for 8 hr (c) and 12 hr (d). Cells were then stained with anti-MICA/B (b and c) or anti-UL16-binding protein 1 (ULBP1) monoclonal antibody (mAb) (d) and analysed by flow cytometry. Black represents the negative control. The % mean fluorescence intensity (MFI) for triplicate cultures was evaluated on the basis of the value obtained with untreated cells (lower panel). His, G.P., and Bor represent histamine, Golgiplug, and bortezomib, respectively. $^{**}P < 0{\cdot}01$ between two values. Values with different letters are significantly different ($P < 0{\cdot}01$).

susceptibility of THP-1 cells to NK cells at E : T ratios of 5 : 1 and 2·5 : 1 (Fig. 8c). Furthermore, the killing of THP-1 cells by purified NK cells was significantly suppressed by anti-MICA/B and anti-ULBP1 (Fig. 8d). No increase in the spontaneous release of the fluorescent probe from histamine-treated THP-1 cells was observed during the cytotoxicity assay (data not shown), indicating that histamine alone did not influence cell viability. Histamine did not affect NK cell-mediated cytotoxicity when included in the 4-hr cytotoxicity assay. These results suggest that the treatment of tumour cells with histamine reduces their susceptibility to attack by NK cells through a decrease in the expression of NKG2D ligands on the cell surface.

## Discussion

Natural killer cells express NKG2D, an activating receptor, at their surface and exhibit cytotoxicity against tumour cells through the recognition of NKG2D ligands such as MIC and ULBPs,[1–3] but the relation between histamine and the NKG2D ligands on tumour cells is unknown. The present study provides evidence that histamine down-regulated the expression of MICA and ULBP1 on THP-1 cells through activation of an H1R-mediated and H2R-mediated ubiquitin–proteasome pathway and consequently reduced susceptibility to NK cell-mediated cytotoxicity. Expression of MICB on THP-1 cells was marginal, as assessed by flow cytometry using anti-MICB mAb, therefore we could not conclude that histamine down-regulates MICB expression in this study.

Natural killer cells are a major source of IFN-$\gamma$.[1] The present study showed that treatment of THP-1 cells with IFN-$\gamma$ augments the expression of MICA/B and ULBP1 and that histamine also down-regulated the augmented expression of NKG2D ligands. These results suggest that IFN-$\gamma$ induces the expression of NKG2D ligands on

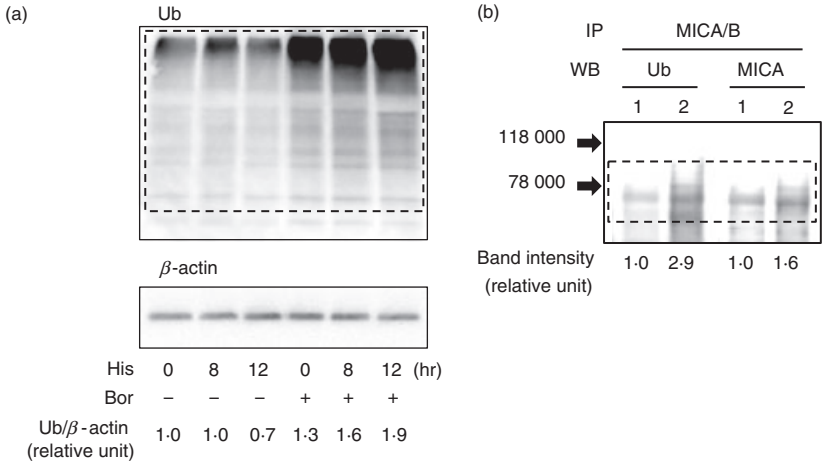



**Figure 7.** Ubiquitination of cellular proteins and MHC class I-related chain A (MICA) by histamine. (a) THP-1 cells were incubated with histamine (His) (100 μM) in the absence or presence of bortezomib (Bor) (100 nM) for the time indicated. Cell lysates were then subjected to Western blotting using anti-ubiquitin (Ub) monoclonal antibody (mAb) or anti-$\beta$-actin polyclonal antibody. The results within dashed line were normalized by the $\beta$-actin level and expressed as relative units versus medium alone. (b) Cell lysates were immunoprecipitated with anti-MICA/B polyclonal antibody and subjected to Western blotting using anti-ubiquitin mAb or anti-MICA polyclonal antibody. Lane 1: THP-1 cells incubated with bortezomib (100 nM) for 8 hr. Lane 2: THP-1 cells incubated with histamine (100 μM) and bortezomib (100 nM) for 8 hr. The results within dashed line were quantified and expressed as relative units versus lane 1. One representative experiment out of four is shown.

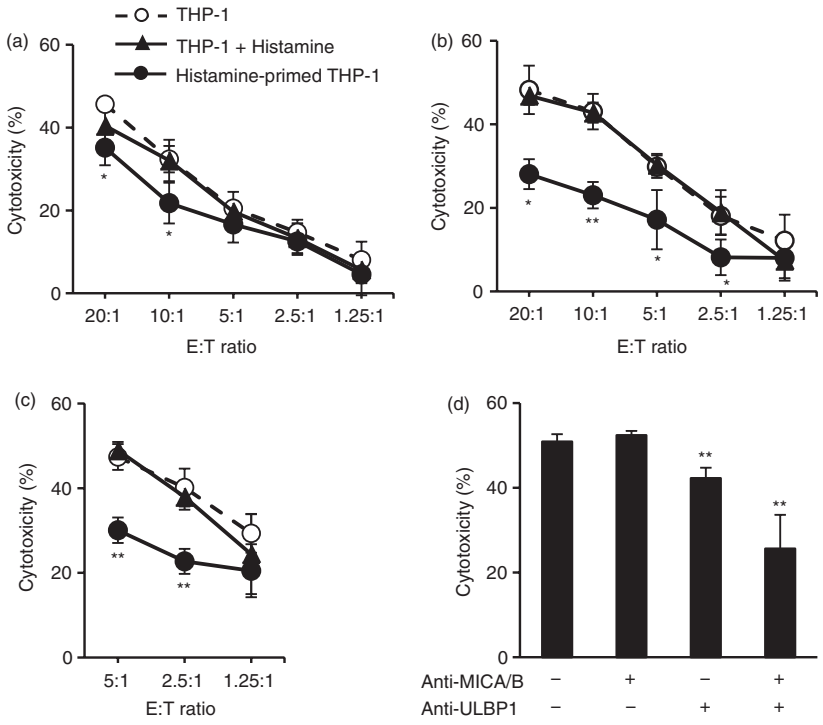



**Figure 8.** Decrease in susceptibility of THP-1 cells to natural killer (NK) cells with histamine. Freshly isolated peripheral blood mononuclear cells (PBMC) (a and b) or purified NK cells (c and d) were used as effector cells. (a–c) THP-1 cells were incubated with medium alone (○) or histamine (100 μM) (●) for 48 hr (a) or 72 hr (b and c), labelled with calcein AM and used as targets at the indicated effector to target (E : T) ratios in a 4-hr cytotoxicity assay. The cytotoxicity assay against untreated THP-1 cells was also performed in the presence of histamine (100 μM) (▲). The results are expressed as the mean ± SD for quadruplicate assays. $*P < 0{\cdot}05$ and $**P < 0{\cdot}01$ compared with untreated THP-1 cells (medium alone). (d) Calcein AM-labelled THP-1 cells were pre-incubated with anti-MHC class I chain A/B (MICA/B) and/or anti-UL16-binding protein 1 (ULBP1) blocking monoclonal antibody (mAb), or control antibody (5 μg/ml each) for 30 min at 37° and used as targets at an E : T ratio of 5 : 1. The results are expressed as the mean ± SD for quadruplicate assays. $**P < 0{\cdot}01$ compared with control antibody (without blocking mAb).

tumour cells and increases susceptibility to NK cell-mediated cytotoxicity and that histamine attenuates this effect. This result is consistent with other reports that IFN-$\gamma$ up-regulated the expression of MIC on monocytes[38] and ULBP1 on acute myeloid leukaemia cells.[39] In line with these observations, histamine down-regulated IFN-$\gamma$ production by T cells through H2R in both human and murine systems,[40] suggesting that histamine attenuates the anti-tumour potential of IFN-$\gamma$. In contrast, recent reports showed that IFN-$\gamma$ down-regulated the expression of MICA and ULBP2.[41,42] This discrepancy is probably linked to the origin and type of tumour and remains to be clarified.

We previously showed that THP-1 cells express H1R and H2R at the mRNA and protein levels.[14] Down-regulation of MICA/B and ULBP1 expression by histamine was reproduced by agonists of H1R and H2R and by A23187 and forskolin. These observations suggest that H1R and H2R on THP-1 cells mediate signalling to down-regulate the expression of MICA/B and ULBP1.

This study showed that histamine did not affect the mRNA expression of NKG2D ligands, indicating that the histamine-induced down-regulation is post-transcriptional. It was reported that the proteolytic shedding of NKG2D ligands is mediated by MMPs such as 'a disintegrin and metalloproteinase' 10 and 17[28–30] or through association with the protein disulphide isomerase ERp5.[31] However, the present study showed that (i) histamine treatment down-regulated the surface expression of ERp5, (ii) inhibition of the MMPs or actin polymerization did not impair the down-regulation, and (iii) soluble forms of MICA and MICB were not detectable even in histamine-treated culture supernatants. A recent report showed that cellular microRNAs suppress MICA and MICB expression through inhibition of translation,[26] but histamine did not affect expression of relevant microRNAs described in the report. These results exclude the possibility that proteolytic shedding, active internalization or microRNAs account for the histamine-induced down-regulation.

The ubiquitin–proteasome pathway plays an essential role in regulating protein levels during the cell cycle, apoptosis, intracellular signal transduction and response to cellular stress.[34,35] Ubiquitination also controls the expression of NKG2D ligands such as MICA[36] and murine ULBP-like transcript 1.[37] The ubiquitin ligase K5

from Kaposi's sarcoma-associated virus-induced ubiquitination of MICA down-regulates its cell surface expression and causes redistribution to an intracellular compartment.[36] This study showed that impairment of protein trafficking by brefeldin A and proteasome activity by bortezomib abrogated the effect of histamine (Fig. 6) and that histamine enhanced poly-ubiquitination of cellular protein and ubiquitination of MICA in the presence of bortezomib (Fig. 7). Taking into account previous reports and the results of this study, it is conceivable that activation of the ubiquitin–proteasome pathway by histamine-mediated signalling is important for the down-regulation of NKG2D ligands. This study observed that bortezomib alone reduced the expression of MICA/B and ULBP1 (Fig. 6c,d), but the reason is unknown.

Previous reports showed that histamine protects NK cells from inhibition by phagocyte-derived reactive oxygen species by maintaining the expression of NKG2D and NKp46 via H2R, which are down-regulated upon the interaction of NK cells with phagocytes.[15,16] Another report showed that interleukin-2-activated human NK cells express H1R and H4R, but not H2R or H3R, and that histamine induces NK cell chemotaxis.[43] However, histamine does not directly affect the cytotoxicity of NK cells themselves.[16,43] Consistent with these findings, our study used freshly isolated PBMC, which included monocytes in the preparation, and purified NK cells and showed that in a 4-hr cytotoxicity assay in the presence of 100 μM histamine, NK cell-mediated cytotoxicity was unchanged.

Histidine decarboxylase is solely responsible for the generation of histamine.[9–11] HDC at the mRNA and protein levels and HDC activity are increased in various human tumours, such as melanoma,[17,18] breast cancers[19,20] and small cell lung carcinoma,[21] and tumour cells expressed H1R, H2R and H4R,[22] indicating that histamine acts as an autocrine growth factor.[18,23] Histamine diminished the proliferation of melanoma cells when acting through H1R, whereas it enhanced the growth of melanomas through H2R.[18] Histamine concentrations are higher in human tumours, such as melanomas and colon and breast cancers, than in the surrounding normal tissue.[44] Median histamine concentrations in colon and breast cancer tissues were reported to be 8·4 μg/g (76 μM), ranging from 0·3 to 20·6 μg/g (2·7 to 187 μM),[45] and 5·4 μg/g (49 μM), ranging from 0·9 to 27·3 μg/g (8·2 to 248 μM),[19] respectively. Therefore, we consider the concentration, 100 μM, used in this study to be comparable to that in human tumours. Furthermore, a recent report showed that histamine from mast cells exhibited growth-inducing activity on human thyroid carcinoma cells.[46] Therefore it is possible that an autocrine histamine from tumours or a paracrine histamine from a local route, such as mast cells, down-regulates the expression of NKG2D ligands and consequently promotes immune evasion.

In conclusion, this study showed that histamine can affect the susceptibility of tumour cells to NK cells by down-regulating the expression of NKG2D ligands. The observation might not be applicable to all tumours, depending on the type and origin of the tumour. However, the results suggest that control of the bioactivity of histamine may be useful for dealing with tumour immunity. Furthermore, NKG2D ligands play important roles in infectious immunity[47] and autoimmunity.[48,49] Therefore, control of histamine activity may be important for the development of treatments for infectious and autoimmune diseases.

## Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research (B) (19390461 to SS) and Young Scientists (B) (21791781 to YN) from the Japan Society for the Promotion of Science, Japan. We thank Dr T. Yamaguchi (Tohoku University Graduate School of Agricultural Science, Sendai, Japan) for providing anti-ubiquitin mAb P4D1. We also thank the Biomedical Research Core of Tohoku University Graduate School of Medicine for the use of its equipment.

## Disclosures

The authors declare no conflict of interest.

## References

1 Bryceson YT, March ME, Ljunggren HG, Long EO. Activation, coactivation, and costimulation of resting human natural killer cells. *Immunol Rev* 2006; **214**:73–91.
2 Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 2003; **3**:781–90.
3 Mistry AR, O'Callaghan CA. Regulation of ligands for the activating receptor NKG2D. *Immunology* 2007; **121**:439–47.
4 Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009; **457**:557–61.
5 Gorh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A* 1996; **93**:12445–50.
6 Jinushi M, Takehara T, Tatsumi T *et al.* Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. *J Immunol* 2003; **171**:5423–9.
7 Cosman D, Mullberg J, Sutherland CJ, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 2001; **14**:123–33.
8 Dy M, Schneider E. Histamine–cytokine connection in immunity and hematopoiesis. *Cytokine Growth Factor Rev* 2004; **15**:393–410.
9 Kahlson G, Rosengren E. New approaches to the physiology of histamine. *Physiol Rev* 1968; **48**:155–96.
10 Schayer RW. Histamine and microcirculation. *Life Sci* 1974; **15**:391–401.
11 Endo Y. Simultaneous induction of histidine and ornithine decarboxylases and changes in their product amines following the injection of *Escherichia coli* lipopolysaccharide into mice. *Biochem Pharmacol* 1982; **31**:1643–7.
12 Endo Y. Induction of histidine and ornithine decarboxylase activities in mouse tissues by recombinant interleukin-1 and tumor necrosis factor. *Biochem Pharmacol* 1989; **38**:1287–92.
13 Yamaguchi K, Motegi K, Kurimoto M, Endo Y. Induction of the histamine-forming enzyme, histidine decarboxylase, in mice by IL-18 and by IL-18 plus IL-12. *Inflamm Res* 2000; **49**:513–9.

14 Minami T, Kuroishi T, Ozawa A, Shimauchi H, Endo Y, Sugawara S. Histamine amplifies immune response of gingival fibroblasts. *J Dent Res* 2007; **86**:1083–8.

15 Hellstrand K, Asea A, Dahlgren C, Hermodsson S. Histaminergic regulation of NK cells. Role of monocyte-derived reactive oxygen metabolites. *J Immunol* 1994; **153**:4940–7.

16 Romero AI, Thoren FB, Brune M, Hellstrand K. NKp46 and NKG2D receptor expression in NK cells with $CD56^{dim}$ and $CD56^{bright}$ phenotype: regulation by histamine and reactive oxygen species. *Br J Haematol* 2006; **132**:91–8.

17 Darvas Z, Sakurai E, Schwelberger HG *et al.* Autonomous histamine metabolism in human melanoma cells. *Melanoma Res* 2003; **13**:239–46.

18 Falus A, Hegyesi H, Lazar-Molnar E, Pos Z, Laszlo V, Darvas Z. Paracrine and autocrine interactions in melanoma: histamine is a relevant player in local regulation. *Trends Immunol* 2001; **22**:648–52.

19 Reynolds JL, Akhter JA, Magarey CJ, Schwartz P, Adams WJ, Morris DL. Histamine in human breast cancer. *Br J Surg* 1998; **85**:538–41.

20 Sieja K, Stanosz S, von Mach-Szczypiński J, Olewniczak S, Stanosz M. Concentration of histamine in serum and tissues of the primary ductal breast cancers in women. *Breast* 2005; **14**:236–41.

21 Graff L, Frungieri M, Zanner R, Pohlinger A, Prinz C, Gratzl M. Expression of histidine decarboxylase and synthesis of histamine by human small cell lung carcinoma. *Am J Pathol* 2002; **160**:1561–5.

22 Boer K, Helinger E, Helinger A *et al.* Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine. *Eur J Cell Biol* 2008; **87**:227–36.

23 Rivera ES, Cricco GP, Engel NI, Fitzsimons CP, Martin GA, Bergoc RM. Histamine as an autocrine growth factor: an unusual role for a widespread mediator. *Semin Cancer Biol* 2000; **10**:15–23.

24 Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 1980; **26**:171–6.

25 Kuroishi T, Kinbara M, Sato N, Tanaka Y, Nagai Y, Iwakura Y, Endo Y, Sugawara S. Biotin status affects nickel allergy via regulation of interleukin-1$\beta$ production in mice. *J Nutr* 2009; **139**:1031–6.

26 Stern-Ginossar N, Gur C, Biton M, Horwitz E, Elboim M, Stanietsky N, Mandelboim M, Mandelboim O. Human microRNAs regulate stress-induced immune responses mediated by the receptor NKG2D. *Nat Immunol* 2008; **9**:1065–73.

27 Cholujová D, Jakubíková J, Kubes M, Arendacká B, Sapák M, Robert MI, Sedlák J. Comparative study of four fluorescent probes for evaluation of natural killer cell cytotoxicity assays. *Immunobiology* 2008; **213**:629–40.

28 Waldhauer I, Goehlsdorf D, Gieseke F *et al.* Tumor-associated MICA is shed by ADAM proteases. *Cancer Res* 2008; **68**:6368–76.

29 Boutet P, Agüera-González S, Atkinson S *et al.* Cutting edge: the metalloproteinase ADAM17/TNF-α-converting enzyme regulates proteolytic shedding of the MHC class I-related chain B protein. *J Immunol* 2009; **182**:49–53.

30 Waldhauer I, Steinle A. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. *Cancer Res* 2006; **66**:2520–6.

31 Kaiser BK, Yim D, Chow IT *et al.* Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. *Nature* 2007; **447**:482–6.

32 MacLean-Fletcher S, Pollard TD. Mechanism of action of cytochalasin B on actin. *Cell* 1980; **20**:329–41.

33 Sciaky N, Presley J, Smith C *et al.* Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *J Cell Biol* 1997; **139**:1137–55.

34 Adams J. The proteasome: a suitable antineoplastic target. *Nat Rev Cancer* 2004; **4**:349–60.

35 Nencioni A, Grünebach F, Patrone F, Ballestrero A, Brossart P. Proteasome inhibitors: antitumor effects and beyond. *Leukemia* 2007; **21**:30–6.

36 Thomas M, Boname JM, Field S, Nejentsev S, Salio M, Cerundolo V, Wills M, Lehner PJ. Down-regulation of NKG2D and NKp80 ligands by Kaposi's sarcoma-associated herpesvirus K5 protects against NK cell cytotoxicity. *Proc Natl Acad Sci U S A* 2008; **105**:1656–61.

37 Nice TJ, Coscoy L, Raulet DH. Posttranslational regulation of the NKG2D ligand Mult1 in response to cell stress. *J Exp Med* 2009; **206**:287–98.

38 Wang H, Ruan Z, Wang Y *et al.* MHC class I chain-related molecules induced on monocytes by IFN-$\gamma$ promote NK cell activation. *Mol Immunol* 2008; **45**:1548–56.

39 Rohner A, Langenkamp U, Siegler U, Kalberer CP, Wodnar-Filipowicz A. Differentiation-promoting drugs up-regulate NKG2D ligand expression and enhance the susceptibility of acute myeloid leukemia cells to natural killer cell-mediated lysis. *Leuk Res* 2007; **31**:1393–402.

40 Horváth BV, Szalai C, Mándi Y, László V, Radvány Z, Darvas Z, Falus A. Histamine and histamine-receptor antagonists modify gene expression and biosynthesis of interferon $\gamma$ in peripheral human blood mononuclear cells and in CD19-depleted cell subsets. *Immunol Lett* 1999; **70**:95–9.

41 Schwinn N, Vokhminova D, Sucker A *et al.* Interferon-$\gamma$ down-regulates NKG2D ligand expression and impairs the NKG2D-mediated cytolysis of MHC class I-deficient melanoma by natural killer cells. *Int J Cancer* 2009; **124**:1594–604.

42 Yadav D, Ngolab J, Lim RS, Krishnamurthy S, Bui JD. Cutting edge: down-regulation of MHC class I-related chain A on tumor cells by IFN-$\gamma$-induced microRNA. *J Immunol* 2009; **182**:39–43.

43 Damaj BB, Becerra CB, Esber HJ, Wen Y, Maghazachi AA. Functional expression of H4 histamine receptor in human natural killer cells, monocytes, and dendritic cells. *J Immunol* 2007; **179**:7907–15.

44 Medina VA, Rivera ES. Histamine receptors and cancer pharmacology. *Br J Pharmacol* 2010; **161**:755–67.

45 Reynolds JL, Akhter J, Adams WJ, Morris DL. Histamine content in colorectal cancer. Are there sufficient levels of histamine to affect lymphocyte function?. *Eur J Surg Oncol* 1997; **23**:224–7.

46 Melillo RM, Guarino V, Avilla E *et al.* Mast cells have a protumorigenic role in human thyroid cancer. *Oncogene* 2010; **29**:6203–15.

47 Kloss M, Decker P, Baltz KM *et al.* Interaction of monocytes with NK cells upon Toll-like receptor-induced expression of the NKG2D ligand MICA. *J Immunol* 2008; **181**:6711–9.

48 Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2003; **100**:9452–7.

49 Eagle RA, Trowsdale J. Promiscuity and the single receptor: NKG2D. *Nat Rev Immunol* 2007; **7**:737–44.